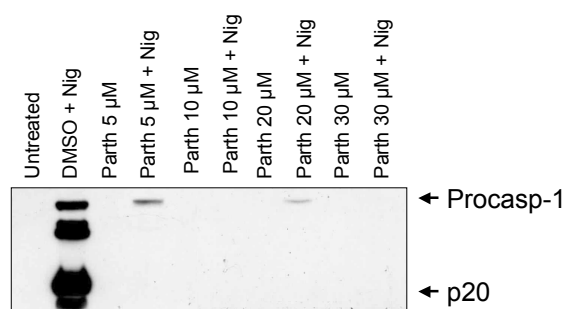


Supplementary Figures 1-6



Supplementary Figure 1. Inhibition of nigericin-induced caspase-1 activation by parthenolide. Immunoblot of caspase-1 in culture supernatants of NG5 macrophages treated with vehicle (DMSO) or increasing concentrations of parthenolide for 15 min followed by nigericin (10 μ M) for 45 min as indicated.

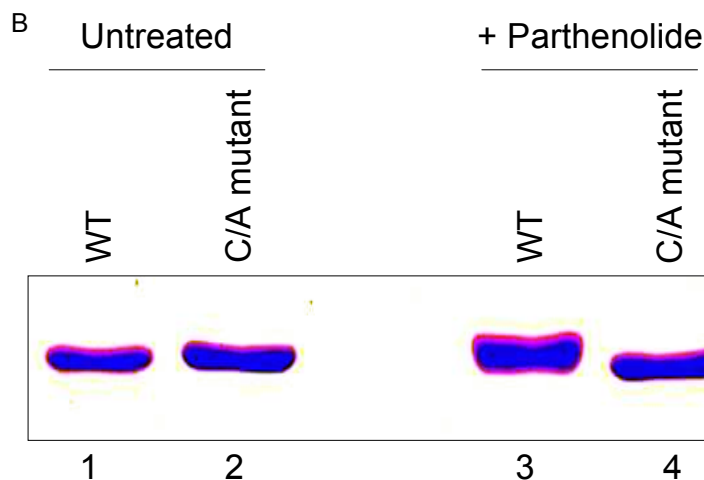
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120                               N PAMPTSSGSE GNVKLCSLEE AQRIWKQKSA
151 EIYPIMDKSS RTRLALIICN EEFDSIPRRT GAEVDITGMT MLLQNLGYSV
201 DVKKNLTASD MTTELEAFAH RPEHKTSDST FLVFMSHGIR EGICGKKHSE
251 QVPDILQLNA IFNMLNTKNC PSLKDKPKVI IIQACRGDSP GVVWFKD

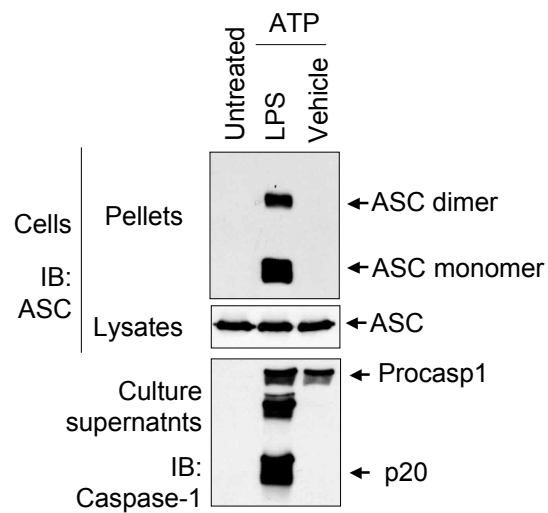
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<u>K. LCSLEE</u> <u>AQR. I</u>	C136 Parthenolide-modified
<u>R. LALIIC</u> <u>NEEFDSIPR. R</u>	C169 Parthenolide-modified
<u>R. EGICGK. K</u>	C244 Parthenolide-modified
<u>K. NCP</u> <u>SLKDKPK. V</u>	C270 Unmodified
<u>K. DKPKV</u> <u>IIQACR. G</u>	C285 Parthenolide-modified
<u>K. VIIQACR. G</u>	C285 Parthenolide-modified

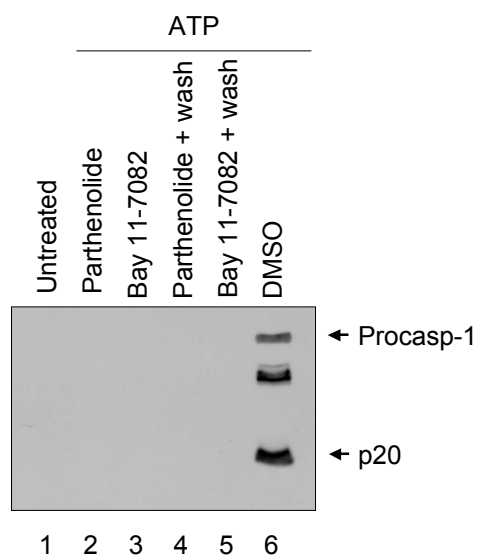


Supplementary Figure 2. Parthenolide covalently alkylate conserved cysteine residues including the active site Cys285 of caspase-1 p20 as determined by mass spectrometric analysis (A), and site directed mutagenesis (B). A (upper panel), sequence of the mature p20 subunit of caspase-1. The red-highlighted regions represent all the peptide sequences covered by mass spectrometric analysis. The lower panel lists all the cysteine containing tryptic peptides that were found to be modified or unmodified by parthenolide.

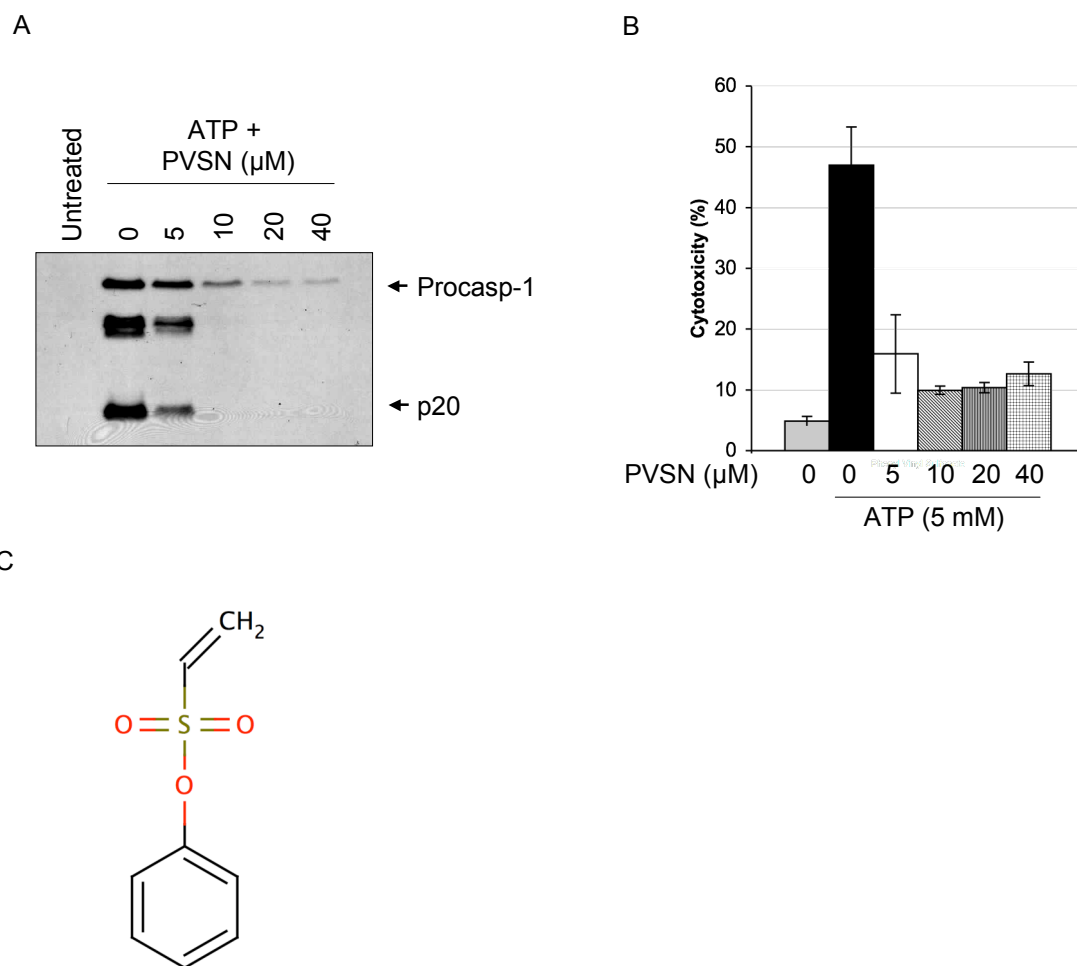
B, Recombinant His6-tagged WT or C/A (C136,169,244,285A) mutant were purified on Talon metal affinity resin and then left untreated or treated with parthenolide (10 μ M), as indicated for 1 h at 37°C. The proteins were then fractionated by SDS-PAGE. Note that the parthenolide-treated C/A mutant p20 (lane 4) do not shift upward as compare to the parthenolide-treated WT p20 (lane 3).



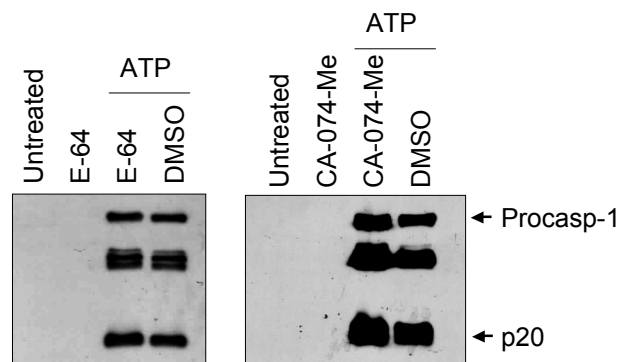
Supplementary Figure 3. Biochemical assay of ASC pyroptosome formation in primary macrophages. Mouse primary BMDM were left untreated, or primed with LPS (500 ng/ml) or vehicle for 5 h followed by ATP (5 mM) for 45 min, as indicated. The culture media were immunoblotted with anti-caspase-1 (lower panel). The cells were lysed and the resulting 6000 rpm cell pellets were cross-linked with DSS. The cross-linked pellets (upper panel) and cell lysates (middle panel) were immunoblotted with anti-ASC antibody.



Supplementary Figure 4. Inhibition of ATP-induced caspase-1 activation by Bay 11-7082 and parthenolide is irreversible. Immunoblot of caspase-1 in culture supernatants of NG5 macrophages treated with vehicle (DMSO), or Bay 11-7082 (10 μ M) or parthenolide (10 μ M) for 15 min as indicated. The cells in lanes 4 and 5 were washed to remove the drugs. All cells in lanes 2-6 were then treated with ATP (5 mM) for 45 min.



Supplementary Figure 5. Inhibition of ATP-induced caspase-1 activation and cell death by phenyl vinylsulfonate. A, Immunoblots of caspase-1 in culture supernatants of NG5 macrophages treated with the indicated concentrations of phenyl vinylsulfonate (PVSN) for 15 min followed by ATP (5 mM) for 45 min. B, Cell death was assayed by measuring the percentages of LDH activity released in culture supernatants of NG5 macrophages (% cytotoxicity) treated with the indicated concentrations of PVSN for 15 min followed by ATP (5 mM) for 30 min. C, Structure of PVSN.



Supplementary Figure 6. Effect of cysteine protease inhibitors on ATP-induced caspase-1 activation. Immunoblots of caspase-1 in culture supernatants of NG5 macrophages treated with the general cysteine protease inhibitor E-64 (100 μ M) or the cathepsin B inhibitor CA-074-Me (40 μ M) for 15 min followed by ATP (5 mM) for 45 min.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Mass spectrometric analysis—To determine the mass shift of a cysteine-containing peptide when modified by parthenolide, parthenolide were mixed with a synthetic cysteine-containing peptide. The peptide control and the peptide after treatment were both subjected to MALDI analysis. The comparison showed complete modification of the synthetic peptide and confirmed that parthenolide modified the cysteine residue in the peptide. The difference in the masses was 248.2, consistent with the monoisotopic molecular weight of parthenolide, 248.14. This value was then implemented in the modification file on the in-house Mascot server, and used in all searches on this project.

Caspase-1 p20 gel bands were excised and destained in 25 mM ammonium bicarbonate in 50% acetonitrile. After destaining, gel pieces were de-hydrated with acetonitrile, reduced with 10 mM DTT (Research Products International) and alkylated with 55 mM iodoacetamide (GE healthcare). Gel pieces were once more dehydrated before being digested with trypsin over night at 37 °C. The Tryptic peptides were extracted twice by 50% acetonitrile and 5% formic acid. The combined peptides were then dried on a centrifuge evaporator and re-constituted in 0.1% trifluoroacetic acid before mass spectrometry analysis.

Chromatographic spotting was performed on an Agilent 1100 series LC system consisting of a G1310A isopump for sample loading, a G1379A degasser, a G2226A nanopump, a G1377A micro-well plate sampler, a G1364D micro-fraction collector, a G1315D diode array detector and a G1316A column switching valve. Mobile A contained 2% acetonitrile and 0.1% trifluoroacetic acid. Mobile phase B contained 98% acetonitrile and 0.1% trifluoroacetic acid. The nano-column used to separate tryptic peptides was house-packed using 3 mm C18 particles (Varian) in a 75 mm x 150 mm fused silica. Samples were loaded onto a 0.3x5 mm C18 trap column (Agilent) at 10 ml/min for 10 min. Peptides were eluted by linear gradient of mobile phase B from 0% to 35% in 60 minutes. The eluted peptides were mixed with matrix through a post-column nano-TEE (Upchurch). The matrix consisted of 5.0 mg/ml CHCA (Sigma) prepared in 70% ACN, 0.1% TFA, 10 mM ammonium monobasic phosphate spiked with 2.5 fmol/ml ACTH 18-39 as an internal standard. The matrix was delivered by a 2500 µl syringe (SGE) on a dual-channel syringe pump (KD Scientific) at 1.2 ml/min. Eluates mixed with matrix were spotted on a blank LC MALDI plate (Applied Biosystems) 10 seconds per spot for a total of 384 spots per chromatogram.

The spotted plate was analyzed a 4800 MALDI TOF/TOF analyzer (Applied Biosystems Inc.). The 4800 6-peptide Cal Mix (ABI) was spotted on all calibration spots for external calibration for both MS and MS/MS mode. MS data were collected in reflector positive mode with 1000 laser shots per spectrum and processed with internal calibration to ACTH (18-39). MS/MS data were collected in 2keV mode with 2000 laser shots per spectrum. A maximum of 10 precursors on each spot were fragmented.

Data were exported to a mascot-compatible format and analyzed against human origin using parthenolide on cysteine, carbamidomethyl on cysteine, and oxidation on methionine as variable modifications. MudPIT ion score cutoff filtering was performed to ensure that all peptide hits had a p value of 0.05 or lower.